Review Article

Current Research & Information on Pharmaceutical Sciences (CRIPS)
@NIPER, SAS Nagar, India

Macroemulsions and Microparticles: Uncovered Mechanistic Insight and Non-conventional Application Potential

Syed Nazrin Ruhina Rahman, Abhinab Goswami, Amoolya Sree, Payel Chakraborty and Tamilvanan Shunmugaperumal*

Departments of Pharmaceutics,
National Institute of Pharmaceutical Education and Research (NIPER)-Guwahati,
Sila Katamur, Changsari, Kamrup, Assam- 781101, India
tamilvanan1@yahoo.co.in

The objectives of this review are (1) to provide the rationale behind choosing micron-level sizing particulate systems like macroemulsions and microparticles, (2) to recollect the presence of two different forms of drug molecules inside the microparticles and to show the utility of eutectic forming excipients for making microcapsules from hydrophilic polymer-based micron-level particles, (3) to present non-conventional application potential of hydrophilic polymer-based micron-level particles and (4) to portray the dispersed oil droplets having a bicompartmental structure in oil-in-water (o/w) macroemulsions along with their possible utility for accommodating multiple cargoes. The potential of making micron sized particles rather than nanometer sized particulate systems is exemplified in a few case studies. The bicompartmental architecture observed in dispersed oil droplets of o/w macroemulsions is a welcome new addition/contribution to emulsion science. This review, therefore, explores the uncovered mechanistic insight and non-conventional application potential related respectively to macroemulsions and microparticles in a non-comprehensive manner.

Introduction

Solid oral dosage forms may be presented as either single units or multiple units. Single units can include soluble and insoluble matrix tablets, coated tablets, or capsules. Multiple units are generally presented as active pharmaceutical ingredients (APIs) (microparticles) or API-loaded beads contained within an outer unit such as a capsule. However, the presentation of the API in multiple unit dosage form provided the following advantages over single unit tablet or capsule dosage form as evidenced by the reported works of different research groups. Multiple unit dosage form for oral use, modifying the dissolution of the API, allows the administration of much smaller API amounts than single unit doses and provides a method of releasing the active ingredients at the desired rate.[1] Multiunit microparticulate dosage forms pass through the gastrointestinal tract avoiding the vagaries of gastric emptying and different transit rates, and, thereby, release APIs more uniformly.[2] Multiple unit system spreads in a large area of absorbing mucosa and prevents exposure to high API concentration when compared to single unit dosage forms on chronic dosing.[3] Compared with single unit dosage forms, multi-particulate oral API delivery systems reduce the risk of systemic toxicity due to dose dumping and local irritation, and ensure predictable gastric emptying.[4] On the other hand, oral colloidal dispersions having macron-or nano-sized dispersed particles encapsulated with a single API molecule can also be made. Being nanosized particles, a lot of advantages such as increased API solubilization, API targeting possibility, multifunctional activity creation, minimizing API toxicity, etc., are evidently being achieved.[5-7]

Unlikely long-or short-term stability problems and thus the potential deprival in the positioning of successfully marketed products are the two major concerns of formulation scientists who involve in

Keywords: Macroemulsion, microparticles, paired bean structure, solid solution, drug crystals, dual drug loading

the development of API-loaded nanometer-sized delivery systems. The examples of such delivery systems include cyclodextrin-based complexes, micelles, nanospheres including nanocapsules and nanoparticles, nanoballoons, nanosized emulsion, liposomes, etc. The nanometer-sized delivery systems are routinely being manufactured by the utilization of somewhat sophisticated size-reduction machinery like homogenizers, microfluidizer and ultrasonicators. Moreover, the addition of specialized multifunctional excipients such as anionic and cationic lipids, liquid and solid polyethylene glycols, nonionic surfactants, natural and synthetic biodegradable polymers, polyoxyethylene group containing synthetic co-polymeric molecules, etc., are necessary to make the delivery systems. Despite the involvement of both multifunctional excipients and sophisticated machinery, the produced so-called nanometer-sized delivery systems are still posing challenges for formulation scientists. The challenges are in terms of desired stability during their production stage and the finished product's self-storage time until their administration into patients. Moreover, each one of the nanometer-sized delivery systems is articulated with special/unique stability-related issues, and these issues are generally overcome by the addition of additional excipient molecules into those systems. For example, the therapeutic oil-in-water (o/w) nanosized emulsions are manufactured by the inclusion of multiple emulsifier molecules. The emulsifiers are usually possessing diverse affinity characteristics: one emulsifier contains aqueous affinity, another one consists of oil affinity and a third one possesses amphipathic/amphoteric characteristics. Whatever the characteristics, the selected emulsifier molecules need to localize at the oil-water interface of the emulsion for stabilizing the dispersed oil droplets against droplet-droplet collision, droplet coalescence, etc. Likewise, more or less similar to the therapeutic nanosized emulsions, the nanocapsules systems are also unequivocally being manufactured by using natural, semi-synthetic and synthetic biodegradable polymers along with oil core and multiple emulsifier molecules. To reduce the auto-oxidation problems associated with oil-, lipid-or nonionic surfactant-based nanometer-sized delivery systems, the antioxidant molecules (either a single or even multiple compounds) such as ascorbic acid, alpha-tocopherol, etc., are purposefully incorporated into these systems as additional excipients. One more popular excipient usually added into the nanometer-sized delivery systems in the earlier time is the antimicrobial agent or preservative. However, its usage is heavily dependent on the route of application of the developed API delivery systems. The use of antimicrobial agents or preservatives is currently restricted for topically applied nanometer-sized drug delivery systems especially if they are meant for ocular use. Taking the issues/problems of long-or short time stability associated with nanometer-sized drug delivery systems into consideration, the formulation scientists look back into the formulation development stage wherein the penultimate step of the nanometer-sized drug delivery systems involved in the production of particles sizes that ranged in the micron-level sizing. In general, the nanometer-level sizing always believes to produce a meta-stable form of particles, and the particles thus produced (due to the involvement of both multifunctional excipients and high-energy size-reduction machinery) are indeed tried to unit together at the expense of smaller (nanometer)-sized particles. In the end, the meta-stable nanometer-sized particles over the time period are slowly converted into stable particles having micron-level sizing. Moreover, this auto-conversion process particularly occurs especially if the surrounding continuous/suspending medium is an aqueous core. The inherent, time-dependent auto-conversion process that occurs within the developed nanometer-sized drug delivery systems thus gives an impetus for formulation scientists to go back (and look) into therapeutic potentials of particulate systems having the stable micron-level sizing particles that are possibly being produced (with the help of low-energy size-reduction machinery) at the particulate production stage itself.

The selected and further discussed micron-level sizing particulate systems in this review include macroemulsions and microparticles. The objectives of the current review are (1) to provide the rationale behind choosing micron-level sizing particulate systems like macroemulsions and microparticles, (2) to recollect the presence of two-different forms of drug molecules inside the microparticles and to show the utility of eutectic forming excipients for making microcapsules from hydrophilic polymer-based micron-level particles, (3) to present non-conventional application potential of hydrophilic polymer-based micron-level particles and (4) to portray the dispersed oil droplets having a bicompartmental structure in o/w macroemulsions along with their possible utility for accommodating multiple cargoes. This review starts with a brief discussion of the rationale for choosing a micron-level sizing particulate system.

Rationale behind choosing micron-level sizing particulate system

The long-or short time stability studies primarily conducted at lab-scale on the micron-and nano-levels sizing particulate systems indicate that the micron-level sizing particulate systems possess somewhat better withstanding capability in terms of both dynamic and kinetic aspects in comparison to the nano-level sizing particulate systems. The complete or partial elimination of a high level of energy input from external size-reduction machinery and possible omission of additional multifunctional excipients incorporation into the drug delivery systems make the micron-level sizing particulate systems more attractive in the sense of lesser manufacturing expenses than the manufacturing expenses associated with the production of nano-level sizing particulate systems which eventually obtained with the help (or sometimes advantage) of both the external size-reduction machinery and additional multifunctional excipients. On the other hand, the clinical or therapeutic efficacies of nano-level sizing particulate systems are well-established through both research publications and the successful positioning of a few commercial products. Unfortunately, the clinical or therapeutic efficacies associated with the micron-level sizing particulate systems are not so well-established and the comparative clinical or therapeutic efficacies study between these two (micro vs. nano level) particulate systems are never the focus of research publications. In other words, the potentiality of nano-level sizing particulate systems is constantly achieved just by comparing them with their drug powder or solution counterparts. No effort or complete ignorance is the case always pending at the researchers' side in terms of comparative clinical or therapeutic (efficacy) experimental studies between micron-and nano-levels sizing particulate systems. The constant/rapid evolution in inter-disciplinary scientific research works results in many outcomes in terms of publications, patents and commercialization which are totally in favor of the nano-level sizing particulate systems that too, are based merely on comparative evaluation between the nano-level sizing particulate systems and their drug powder or solution counterparts. But in terms of possible reduction in the production cost, improved final product stability and consumer affordability, it is the right junction to ask/put the question, "Why we aren't switching back to the production of less-expensive micron-level sizing particulate systems, if at all, these products are able to show rigorously the clinical or therapeutic efficacies that are similar or slightly lower or better compared to the clinical or therapeutic efficacies afforded by the nano-level sizing particulate systems for the management or treatment of particular syndrome". The switching-back process additionally renders the advantages of producing less-expensive products without compromising the clinical or therapeutic efficacies for the management or treatment of a particular syndrome. This review will try to cover the experimental work performed at a laboratory scale using the micron-level sizing particulate systems (macroemulsions and microparticles) for the management of a few symptoms like eradication of inflammation causing microbes in chronic periodontal disease or Acne vulgaris disease condition and acute or chronic pain associated with the primary dysmenorrheal condition.

Uncovered mechanistic insight and non-conventional application potential of microspheres/microparticles

When the solid APIs are incorporated into matrix/particulate forming hydrophobic or hydrophilic polymer and molten oil or lipid excipients, there are two modifications will occur on the physical states of solid APIs: molecular dispersion/solution and simple drug crystal formation within the developed matrix/particulate structure. If the molecular dispersion/solution formation occurs between the API and matrix/particulate forming excipients, then, the API is likely to be released rapidly from the matrix/particulate system at the time of dissolution test. The matrix/particulate structure also appears as smooth-surfaced when observed using electron (scanning or transmission) microscopic techniques. Conversely, the occurrence of drug micro-or nano-crystal formation within the matrix/particulate structure results in the retardation of drug release at the dissolution testing time and further leads to the creation of rough-surfaced structure on the developed matrix/particulate system when visualized under these two electron microscopic techniques. Irrespective of the physical forms of the final products (either liquid-retentive or solid free-flowing), the generated matrix/particulate structure shows the presence of either the molecular dispersion/solution or the drug micro-or nano-crystal within it and this structural discrimination is all depending on the APIs physicochemical properties, matrix/particulate forming excipients selected and manufacturing process conditions/parameters. For instance, the API is in dissolved form within the

polymer solution and the microencapsulation used is a spray drying technique, then, the final microsphere structure is of matrix or monolithic (or molecular or solid solution) type. Conversely, if the API is in dispersed form within the polymer solution and the same spray drying technique is employed, then, the final product belongs to the drug reservoir (sometimes called microcapsules) type wherein the API molecules are simply embedded in a well-defined polymeric wall structure.

At the very outset, the microparticles are multiple-unit drug delivery systems intended primarily for the oral route of administration. Later on, the parenteral injections are developed based on microparticles prepared from biodegradable polymers. The main functions of the microparticles for oral drug delivery are to avail taste-masking of bitter or salty APIs and to obtain sustained drug release of APIs for reducing the dosing frequency. For parenteral injectable microparticles, the main advantage is to control the API release over the time periods of days, weeks, months or even years. The controlled/ sustained release of incorporated API from the microparticles is one of the main reasons why the microparticles are generated for both oral and parenteral uses. Concerning the utility of microparticles for topical (percutaneous and buccal cavity) administration, it becomes necessary to incorporate the generated hydrophobic and/or hydrophilic polymers-based microparticles into a semi-solid carrier system, called gels or ointment base, so that the solid free flowing microparticulate structure will reside inside the semi-solid base when applied topically onto the patient's skin or teeth surfaces. Whatever it may be the routes (oral, parenteral and topical) of administration and the types of polymers (hydrophobic and/or hydrophilic) used, the internal structure has a profound influence on the final performances of drug-laden microparticles at both in vitro and in vivo conditions. The internal structure here indicates simply the presence of API either in molecular dispersion/ solution state or in micro-or nano-crystal state inside the generated hydrophobic and/or hydrophilic-based microparticles. Again, the above-said two different states of API within the microparticle structure rely heavily on the API's initial loading into the microparticles. The consequences due to the presence of drug molecular dispersion/solution and micro-or nano-crystal within the microparticles are exemplified below in two different case studies.

Case study-1: Indomethacin and Ibuprofen-loaded polystyrene microparticles

Following oral ingestion, the controlled/sustained release single-unit dosage forms such as tablets or capsules keep their integrity starting from the stomach and small intestine to the large intestine. These single-unit dosage forms also show variations in gastric emptying time value and thus the diversified transit rate at different parts of the gastrointestinal tract. Moreover, these single-unit systems always associate with the risk of dose dumping leading to local and systemic side effects due to the sudden rise in drug concentrations. On the other hand, multiple-unit dosage forms like matrix (microspheres, microparticles) or reservoir (microcapsules) and coated beads are quite free from these problems. Because after administration, they pass through the gastrointestinal tract smoothly and uniformly like a solution that results in a uniform drug release from the multiple-unit dosage forms and thus ensures the absorption of the drug throughout the gastrointestinal tract.[2-4] They also reduce any irritation or local side effects caused by local high concentrations of drugs. Matrix systems (microspheres, microparticles) are selected rather than reservoir systems (microcapsules) and coated beads because of the ease of manufacture and because of the relatively high loading doses required. To make oral microparticulate dosage forms for nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and ibuprofen, the biocompatible and hydrophobic polymer, polystyrene was selected. For a poorly soluble drug like ibuprofen, the relatively high-loading doses usually lead to bulky dosage forms, particularly with respect to reservoir systems.

The internal structures of these two developed microparticles loaded with indomethacin and ibuprofen were mechanistically analyzed as a function of drug loadings using scanning electron microscopy. The variation in drug loadings from 20 to 70 % w/w did not produce any perceptible change in the shape of indomethacin-loaded polystyrene microparticles (Figure 1A). However, the appearance of some roughness on the microparticle's surface was seen at 70 % w/w drug loading (Figure 1A) which could predict the drug crystal formation inside the microparticles. On the other hand, the shape of ibuprofen-loaded polystyrene microparticles was found to be influenced by the drug loadings (Figure 1B). When the drug loadings were less than 50 % w/w, the microparticles were spherical, above this drug loadings, the microparticles become irregular in shape. If a drug is completely soluble in the polymer solution, a pressure difference between the disperse phase and the continuous phase exists,

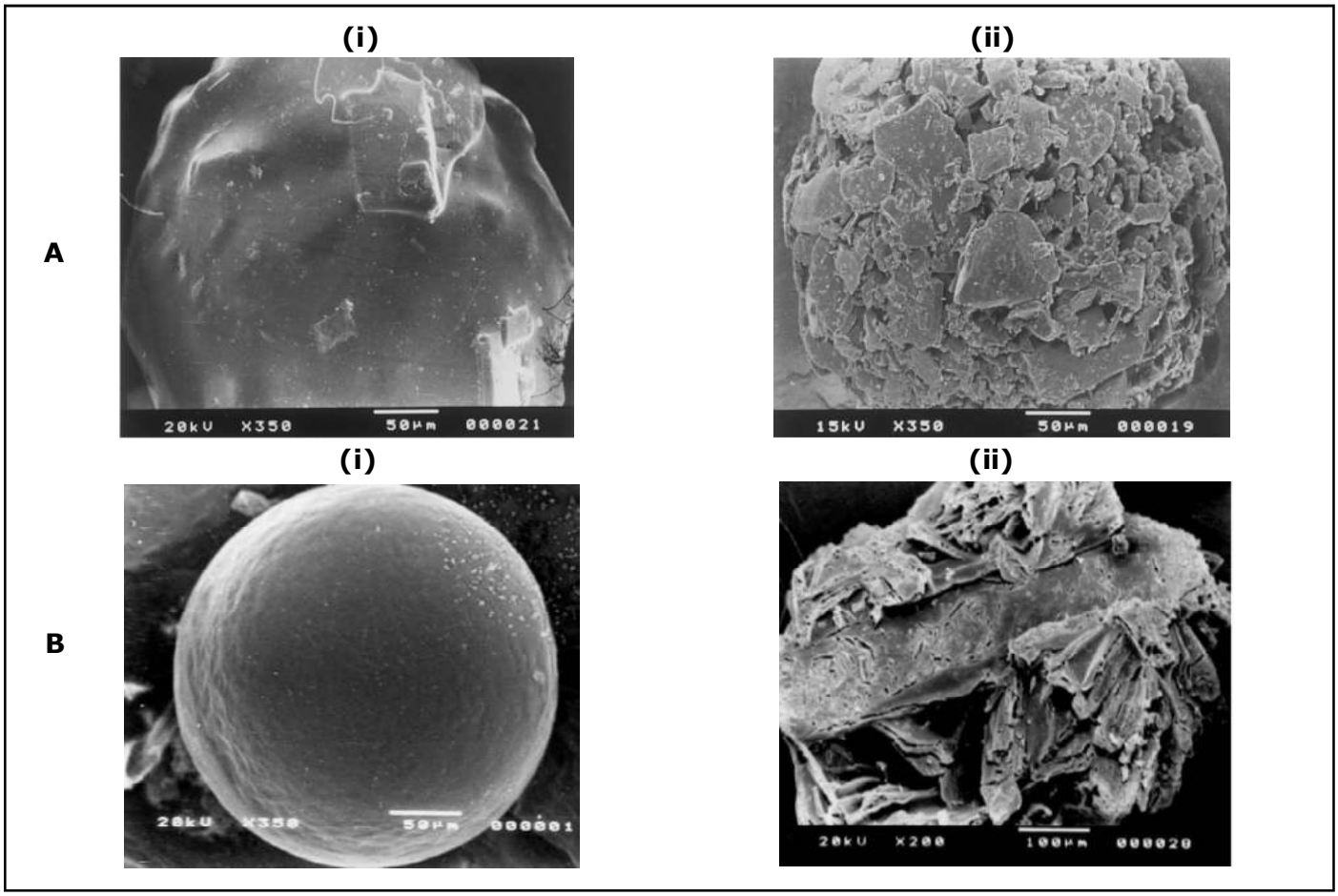

Figure 1. Scanning electron micrographs of polystyrene microparticles loaded with (A) indomethacin (i. 20 & ii.70 % w/w) and (B) ibuprofen (i. 20& ii.70 % w/w).

and the greater the pressure difference, the greater the distortion of the microparticles.[8] When ibuprofen loadings were less than 50 % w/w, the pressure difference between the two phases was not high enough to distort the spherical shape of the particles. Above these drug loadings, a considerable pressure difference between the two phases would have caused distortion of shape.

3.2. Case study-2: Development of an adjunct (formulation) to non-surgical periodontal therapy in the treatment of chronic periodontitis (Ornidazole-loaded polyethylene glycol-based microspheres)

Chronic periodontitis is an oral disease condition that occurs in teeth and their supporting structures.[9] Formation of pockets or pathologically deepened sulcus, leading to loss of teeth and destruction of tooth-supporting structures, caused by periodontal-pathogenic bacterial infections accompanied with sub-gingival plaque. Periodontal disease is also caused due to variation occurring in the microflora, histopathological variations, clinical symptoms and the location of the inflammation leading to deepened sulcus.[10] The early/moderate stage of periodontitis is termed "gingivitis" which is characterized by swelling, light bleeding and redness of marginal gingiva and in case chronic periodontitis and/or the final stage alveolar bone resorption occurs and supporting structures such as ligaments start detaching from teeth. About 80 % of the world's population is suffering from moderate to severe periodontitis. Treatments such as scaling and root planing are the options to prevent periodontal disease conditions.

Scaling is a process that comprises of elimination of calculus, plaque, tooth deposits and some stains. It can be done either above the gum (supra-gingival) or below the gum (sub-gingival). Root planing is a more radical treatment that involves the elimination of necrotic substances from the surface of the tooth under the gum, otherwise, it is similar to scaling. Undergoing scaling and root planing treatments only prevent the effects of periodontitis, it cannot clear the pathogenic bacteria colonizing in sub-gingival and supra-gingival spaces. Therefore, many drug delivery systems have been developed recently to cure periodontal diseases such as injectable drug delivery, bioadhesive gels, and adjunctive therapy which involves the application of various drugs-

Table 1. *In vitro* release profiles comparison for ornidazole (ORN) powder, ORN-loaded microspheres and ethylcellulose (EC)-coated ORN microspheres (Mean ± S.D).

Time (mins)	Cumulative percentage drug release from		
	ORN powder (mean ± SD, n=3)	ORN microspheres (mean ± SD, n=4)	EC-coated ORN microspheres (mean ± SD, n=3)
5	0.209 ± 0.365	0.155 ± 0.31	0.57 ± 0.03
15	59.94 ± 12.32	28.48 ± 11.75	7.31 ± 3.1
30	72.42 ± 18.18	56.24 ± 13.839	24.38 ± 5.5
45	100.64 ± 9.222	67.77 ± 7.328	41.45 ± 7.7
60	Not applicable	73.15 ± 5.04	42.25 ± 10.12

Table 2. Topical gels ingredients.

Ingredients	Formulation (% w/v) code					
	F*	F1	F2	F3	F4	F5
Ornidazole (ORN) drug powder	0.1	-	-	-	-	-
Ornidazole (ORN) microspheres or ethylcellulose (EC)-coated ORN microspheres	-	0.1	0.1	0.1	0.1	0.1
Carbopol 934	2	1	2	4	5	-
Hydroxy Ethyl Cellulose (HEC)	-	-	-	-	-	2.5
Distilled water	q.s	q.s.	q.s.	q.s.	q.s.	q.s

F* = control formulation containing only drug powder

incorporated microspheres, systemic antibiotics as well as antibiotics in combination with drugs.

Ornidazole (ORN) is a 5-nitro imidazole derivative with antiprotozoal and antibacterial activity against pathogenic anaerobic bacteria. Its antimicrobial activity is due to the reduction of the nitro group to a more reactive amine that attacks microbial DNA, inhibiting the further synthesis and causing degradation of existing DNA. Therefore, systemic and/or local application of ORN microspheres along with scaling and root planning treatments might be effective in the inhibition of colonial growth of patho-periodontic anaerobic bacteria so as to prevent and cure periodontal disease conditions. The ORN-loaded polyethylene glycol-based microspheres meant for topical delivery is more likely to provide the following two advantages (1) a better-controlled delivery of drug from ORN microspheres coated with or without ethylcellulose and after incorporation of drug-loaded microspheres into carbopol or hydroxyethyl cellulose-based gel formulation and (2) obtaining an adjunctive therapy thus preventing surgical procedure such as scaling and root planning over tooth cavity.

Adjunct/Adjunctive treatment is defined as another treatment that is carried out along with the primary treatment. Its purpose is to assist the primary treatment and is therefore generally called an "adjunct". Here, scaling and root planing are considered as a primary treatment option for chronic periodontitis whereas topical gels containing ORN-loaded hydrophilic polymer-based microspheres act as non-surgical periodontal adjunctive therapy. By combining both primary and adjunct therapies together, it is likely that the destruction of teeth-supporting ligament structure by pathogenic

microorganisms will be eradicated effectively. The microspheres were prepared based on two hydrophilic pharmaceutical excipients: polyethylene glycol and chitin. Interestingly, these two excipients have two different characteristics when they are used to develop injectable micro-and nano-formulations. Again, the preformed microspheres were coated with ethylcellulose (EC) polymer to make the ORN-loaded and EC-coated microspheres. To prepare the EC-coated microspheres, weighed amounts of ORN-loaded microspheres and EC were mixed in a ratio of 2:1. Using the TLC spraying apparatus, 20 ml of cyclo-hexane was sprayed onto the mixture containing the microspheres and EC. The resulting coated EC-microspheres were kept in the open air for drying over the time period of 12 hours.[11] Table 1 shows in vitro release profiles of ORN drug powder, ORN-loaded microspheres and EC-coated ORN-loaded microspheres. From Table 1, it is clear that coating of the microspheres with EC always showed the retardation of ORN release and incorporation of ORN into microspheres resulted in release retardation in comparison to ORN drug powder at all the studied dissolution time periods (5-60 minutes). Finally, the ORN powder and ORN-loaded microspheres with or without EC-coating were incorporated into two different gel bases as shown in Table 2.

Since the microspheres were prepared based on two hydrophilic polymers (PEG and chitin), it is more likely that a sufficient amount of ORN will be released initially from the microspheres into the gel base (due to the presence slightly hydrophilic structure of the selected gel base) and subsequently, the released drug will be available and even sufficient to act on the pathogens present over the applied buccal (gum) area instantly. This type of instant release of sufficient drug amount provides the prevention of the pathogens forming a buccal biofilm (association of single microbes into a colonized and non-penetrable form) that is more resistant to drug treatment and thus necessitate the treatment options such as scaling and root planing. If the hydrophobic polymers (instead of hydrophilic polymers) are used to prepare microspheres, then, the drug release is not to be instant (may be imminent) to reach the gel base. On the other hand, the question of using the drug incorporated in the gel base (rather than incorporating the drug-loaded hydrophilic polymer-based microspheres into the gel base) is again possible but we cannot expect the same/similar instant release of sufficient drug amount from the highly hydrophobic gel base only formulation. Here too, the question of selecting the hydrophilic or emulsion type gel base is possible and although a similar instant release of sufficient drug amount from the base can be achieved, the obtaining of a controlled drug release profile is not possible and the prepared hydrophilic gel-based formulation needs to be applied frequently into the buccal cavity. Usually, patients having periodontal disease condition are advised to use the drug-loaded gels at night time and the frequent application during the night time has thus associated with the problem of sleep deprivation. Hence on account of preventing the pathogenic microbes to become forming biofilm (or even calculus, plaque, tooth deposits and some stains) over the teeth area, a formulation strategy of providing initial instant release followed by controlled drug release could be a better option to act as an adjunct (formulation) to non-surgical periodontal therapy in the treatment of chronic periodontitis. And the results to show and fulfill the above-said initial instant release followed by controlled drug release via the currently proposed gel(s) containing the ORN-loaded hydrophilic polymer-based microspheres was tested via in vitro permeation study.

A previously reported method by Tamilvanan and Baskar (2013) to investigate the release of celecoxib from oil-in-water nanosized emulsion was followed to see in vitro release/permeation of ORN from drug-loaded gel and gel containing ORN microspheres or EC-coated ORN microspheres for 60 minutes using Franz diffusion cells.12 After incorporation into the carbopol or hydroxyethyl cellulose (HEC)-based gel, the ORN-loaded microspheres dissolved within 10 mins whereas EC-coated ORN microspheres showed very good stability in all the formulations up to over the time period of 30 to 45 minutes. As expected, all the gels containing ORN microspheres (formulation codes from F1 to F5) possessed the percentage drug permeation/release values in a phosphate buffer solution of pH 6.8 (Table 3) that were significantly lower when compared to the value (3.63 ± 0.34) obtained for gels containing ORN drug powder only (F*). Between the gels containing ORN microspheres and EC-coated ORN microspheres, the calculated value of percentage drug permeation/release was, however, found to be low for the formulation containing EC-coated ORN microspheres (Table 3). This indicates the EC coating onto the ORN microspheres had higher retardation of ORN release/permeation. A similar trend in the cumulative permeation percentage values was noticed for the gels containing ORN powder and ORN microspheres or EC-coated ORN microspheres when the permeation

Table 3. In vitro cumulative permeation percentage values obtained in a phosphate buffer solution of pH 6.8 for the gel formulations containing ornidazole (ORN) drug powder, ORN microspheres and ethylcellulose (EC)-coated ORN microspheres.

Formulation Code	Time (minutes)	Cumulative permeation percentage value obtained from gels containing		
		ORN drug powder (denoted as F*) (Mean ± S.D, n=3)	ORN microspheres (Mean ± S.D, n=3)	EC-coated ORN microspheres (Mean ± S.D, n=3)
F1	5	Not determined	1.688 ± 1.51	0.430 ± 0.58
	15	Not determined	5.676 ± 1.37	1.107 ± 0.78
	30	Not determined	12.62 ± 6.37	2.138 ± 0.51
	45	Not determined	18.277 ± 5.48	13.859 ± 1.50
	60	Not determined	24.120 ± 3.00	21.041 ± 2.10
F2	5	2.888 ± 2.51*	4.729 ± 2.80	0.256 ± 0.04
	15	7.676 ± 1.45*	7.131 ± 3.14	1.023 ± 0.15
	30	14.72 ± 5.37*	14.186 ± 3.40	5.493 ± 0.87
	45	22.677 ± 7.48*	16.724 ± 2.66	17.317 ± 1.63
	60	38.120 ± 3.00*	18.563 ± 2.11	18.618 ± 0.98
F3	5	Not determined	1.208 ± 0.44	0.267 ± 0.03
	15	Not determined	2.170 ± 0.55	1.942 ± 0.19
	30	Not determined	4.734 ± 2.37	4.035 ± 1.12
	45	Not determined	9.904 ± 1.01	7.843 ± 0.64
	60	Not determined	13.036 ± 1.07	9.867 ± 0.35
F4	5	Not determined	1.197 ± 0.26	1.206 ± 0.29
	15	Not determined	3.277 ± 0.93	2.181 ± 0.59
	30	Not determined	6.779 ± 2.77	4.729 ± 0.31
	45	Not determined	8.983 ± 2.76	5.514 ± 0.36
	60	Not determined	12.377 ± 1.88	8.811 ± 0.83
F5	5	Not determined	1.44 ± 0.19	0.741 ± 0.39
	15	Not determined	1.77 ± 0.56	1.585 ± 0.34
	30	Not determined	4.801 ± 1.21	2.865 ± 0.32
	45	Not determined	6.317 ± 2.30	4.197 ± 0.28
	60	Not determined	10.665 ± 3.21	5.444 ± 0.25

* Particular gels (denoted as F*) prepared from carbopol base (2.5% w/v) only and it contains only 100 mg ORN powder alone (one gram of gel containing 25 mg drug was taken for this study)

medium was changed from phosphate buffer solution of pH 6.8 to an artificial saliva solution of pH 6.8 (Table 4).

Case study-3: Influence of eutectic liquid on hydrophilic polymer-based micro systems

Multiple unit formulations consist of both monolithic (microspheres or microparticles) and reservoir (microcapsules) types. Whereas the monolithic type possesses an inseparable polymer and drug matrix, the presence of a distinct polymeric wall structure with a central drug core is inevitable for the reservoir type. With the help of protective colloids such as polyisobutylene (PIB), reservoir type particulate systems (microcapsules) are routinely being made.[13-15] But the PIB did not work when polyethylene glycol (PEG) was used to make reservoir

Table 4. In vitro cumulative permeation percentage values obtained in artificial saliva solution of pH 6.8 for the gel formulations containing ornidazole (ORN) drug powder, ORN microspheres and ethylcellulose (EC)-coated ORN microspheres.

Formulation Code	Time (minutes)	Cumulative permeation percentage value obtained from gels containing		
		ORN drug powder (denoted as F*) (Mean ± S.D, n=3)	ORN microspheres (Mean ± S.D, n=3)	EC-coated ORN microspheres (Mean ± S.D, n=3)
F1	5	Not determined	5.559 ± 0.32	1.814 ± 0.27
	15	Not determined	8.562 ± 0.63	5.392 ± 0.33
	30	Not determined	13.833 ± 0.65	9.696 ± 0.32
	45	Not determined	20.620 ± 0.66	19.614 ± 0.52
	60	Not determined	28.326 ± 0.64	24.229 ± 0.99
F2	5	4.87 ± 2.51*	0.967 ± 0.32	1.176 ± 0.34
	15	8.76 ± 1.45*	2.084 ± 0.43	1.869 ± 0.12
	30	15.64 ± 5.37*	3.047 ± 0.11	5.860 ± 0.51
	45	24.38 ± 7.48*	5.932 ± 0.29	7.978 ± 0.26
	60	38.12 ± 3.00*	6.100 ± 0.42	8.255 ± 0.98
F3	5	Not determined	2.082 ± 0.45	0.995 ± 0.25
	15	Not determined	3.895 ± 0.17	1.639 ± 0.54
	30	Not determined	8.242 ± 0.33	6.836 ± 0.59
	45	Not determined	13.691 ± 0.69	11.469 ± 0.57
	60	Not determined	16.469 ± 0.73	14.199 ± 0.42
F4	5	Not determined	2.082 ± 0.45	1.007 ± 0.06
	15	Not determined	2.923 ± 0.33	2.404 ± 0.50
	30	Not determined	7.941 ± 0.20	6.886 ± 0.33
	45	Not determined	10.195 ± 0.32	9.063 ± 0.21
	60	Not determined	11.326 ± 0.32	9.956 ± 0.19
F5	5	Not determined	1.567 ± 0.34	1.384 ± 0.16
	15	Not determined	3.725 ± 0.34	7.529 ± 0.28
	30	Not determined	6.166 ± 0.35	6.014 ± 0.17
	45	Not determined	8.79 ± 0.22	7.633 ± 0.35
	60	Not determined	12.5 ± 0.54	13.877 ± 0.29

* Particular gels (denoted as F*) prepared from carbopol base (2.5% w/v) only and it contains only 100 mg ORN powder alone (one gram of gel containing 25 mg drug was taken for this study)

type particulate systems and the produced particles appeared as monolithic type. With a view to find out an alternative to PIB in producing reservoir type particulate systems from PEG, Tamilvanan and Chanda (2019) have used a eutectic liquid consisting of a 1:1 ratio of camphor and menthol.[16] The peculiarity of developed micron-level particulate systems is the change in morphological behavior in the presence of surrounding aqueous medium (Figure 2). There are two different models described to indicate the change in morphological behavior of micron-level particulate systems on contact with an aqueous medium. While the first model describes the traditional way of dissolution medium permeation into the hydrophilic PEG matrix, the second model proposed is completely related to the participation/ presence of eutectic liquid in micron-level systems. Since both eutectic liquid and ORN are hydrophobic in nature, the ORN prefers to mix with eutectic liquid rather than in the PEG molecules. Indeed, it is reflected in the ORN entrapment efficiency value wherein the eutectic liquid plays important to achieve high entrapment of ORN (almost 15-20 % more) into the micro systems.[16]

Uncovered mechanistic insight of macroemulsions

It was around more than 50 years that colloidal drug delivery systems are being designed. Enhanced

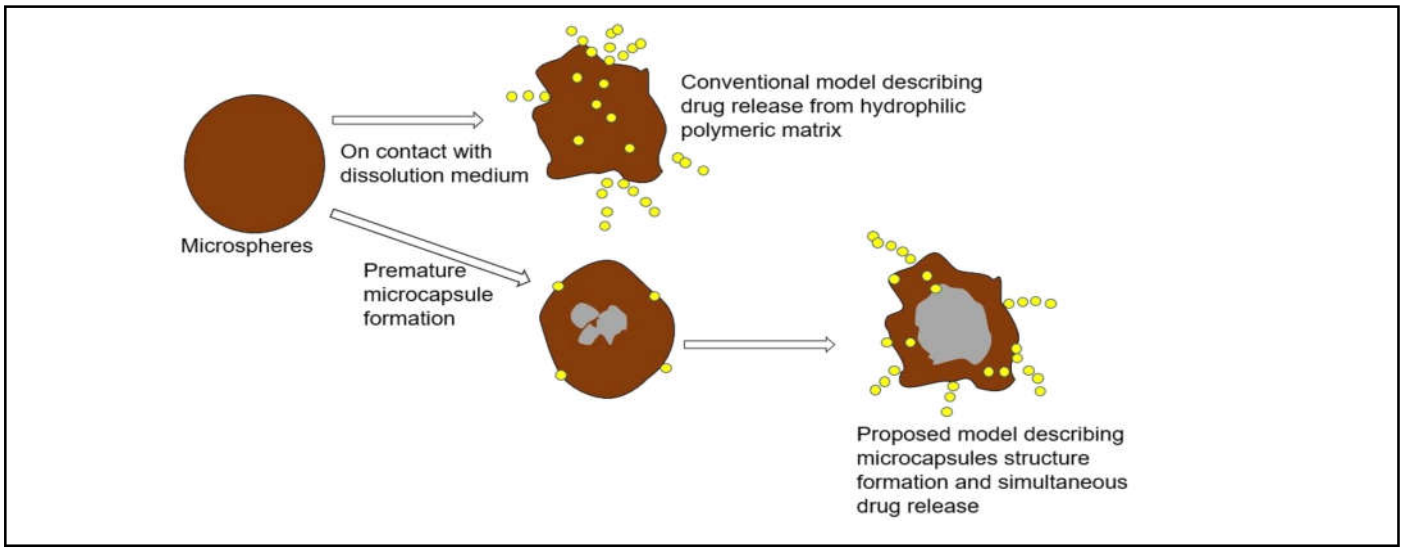

Figure 2. Proposed model describing the attitude of micron-level particles under dry and wet conditions

aqueous solubility value and elevated GI epithelial transport mechanism of hydrophobic drug molecules are easily achieved by using colloidal dispersions like cubosomes, ethosomes, liposomes, nanospheres, nanocapsules, niosomes, transferosomes, etc. In this list, the o/w emulsions possess the ability to hold both hydrophobic and hydrophilic drug molecules depending on solubility enhancement or GI epithelial transport elevation.[17,18] Between these two emulsions, the o/w emulsions have attractive multifunctional activities for medical and pharmaceutical applications. Even a sizeable number of commercial products are launched to correct various infectious diseases and reduce the myriad of symptoms related to the diseases.[19,20] Strictly speaking, the commercially available emulsions consist of dispersed oil droplets having a mean particle size ranging from 300 to 500 nm level. In general, the nano-sized particles produce a meta-stable form of particles due to the involvement of both multifunctional excipients and size-reduction machinery. The meta-stable nano-sized particles over the time period are slowly auto-converted into the stable particles having micron-level sizing i.e., macroemulsions.

Apart from incorporating the hydrophobic drug molecules into the emulsion dispersion system, it is also interesting to incorporate the water-soluble drug molecule into the dispersion system along with the hydrophobic drug moieties. Teixeira et al., in the year 2000 developed cationic emulsions consisting of the dispersed medium chain triglyceride (MCT) droplets stabilized with the three-different emulsifiers (Lipoid-E80, poloxamer-188 and stearylamine).[21] A peculiar 'handbag' architecture was found with the dispersed MCT droplets which is probably the first report of bi-compartmentalization within the emulsion system. By playing with an oil combination and a single emulsifier molecule, Leonardi et al., in the year 2015 created bicompartmental architecture on the dispersed oil droplets of o/w nanosized emulsions.[22] In the year 2019, Puri et al., developed the bicompartmentalized oil droplets by changing the ratio of two-different non-volatile oils and tried for the first time to entrap ginger powder, especially at one part of the compartment leaving another part for other hydrophilic drug.[23] Since then, the application of the dual drug-loading concept in the emulsions having bicompartmentalization on the dispersed oil droplets becomes a fascinating research work. Various terminologies have been ascribed to denote bicompartmentalization which include anisotropic, handbag, Janus and paired-bean.[24, 25]

Through the currently proposed o/w macroemulsions containing the bicompartmentalization on the dispersed oil droplets and prepared by low-shear size-reduction machinery, the following two advantages are being expected (1) the possibility to entrap two-different drug molecules possessing diverse physicochemical properties but similar therapeutic activity and (2) obtaining a synergistic therapeutic effect thus preventing the multiple time administration/application of the developed formulation for the treatment of disease like melanoma, for the management of inflammation produced due to Acne vulgaris and for the diminishment of pain severity during the primary dysmenorrheal condition.

Macroemulsion formulation development and characterization

With the help of laboratory scale mixing equipment

such as electric and magnetic stirrers, the o/w emulsions comprising bicompartmentalization on the dispersed oil droplets (micron-sized) can easily be made to meet the industrial aspirations of making a liquid-retentive topical macroemulsions for the treatment of skin melanoma, for the management of inflammation produced due to Acne vulgaris and for the diminishment of pain associated with the primary dysmenorrheal condition. Before making the macroemulsions, the selected drug molecules need to be processed either by a modified vapor pressure diffusion method or an anti-solvent precipitated method.[26, 27] The examples of drug molecules include adapalene (ADP), curcumin (CUM), fenugreek (FEN) or ginger (GIN). Since ADP possesses a highly fragile or unstable structure upon exposure to light and other processing conditions, unprocessed ADP drug particles are used throughout this study. The modified vapor diffusion method and anti-precipitation technique are utilized to prepare the processed samples for CUM whereas for the FEN and GIN, only the anti-solvent precipitation technique is applied. The selected non-volatile oils along with or without drug molecules may be mixed with a water phase containing single emulsifier molecules. Initially, the macroemulsions are prepared using synthetic surfactants (Tween 20 or Tween 80) and the formed emulsions contain the dispersed oil droplets with paired bean structures. The macroemulsions can also incorporate a phytomedicinal compound possessing the flavonoids/ polyphenolic structures like CUM, quercetin, rutin, GIN and FEN not only for their potential health benefits but also for their capability to position at the o/w interface and thus diminishing the interfacial tension between oil and water. The macroemulsions prepared with the amalgamation of the CUM crystals made by the modified vapor-diffusion method or with the incorporation of nanosized CUM particles prepared by the anti-solvent precipitation method are also able to produce a liquid-retentive emulsion containing the few paired bean-structured dispersed oil droplets (Figure 3A). The observed peculiar paired bean structure may also be further discriminated by incorporating another phytomedicinal compound (like asafetida or the lipophilic dye such as 6-coumarin having pale orange/very dark orange color) during the emulsion preparation step itself. So, the presence of paired bean structure might unequivocally be seen with two different colors provided by two different combinations of phytomedicinal compounds (CUM and asafetida, FEN and GIN or 6-cumarin). This invention thus indicates the distinguished properties of the paired bean structure having varied affinity to incorporate the medicinally-valued compounds derived from plant sources. Additionally, an anti-acne therapeutic agent such as ADP is also incorporated in its unprocessed form into the paired bean structure of the macroemulsions.

The typical formulas used to make macroemulsion consists of ADP, CUM, FEN and GIN are shown in Table 5. The mean particle diameters of the dispersed oil droplets of the macroemulsions were determined by using a Malvern master-sizer (Malvern, instrumentation limited; London, UK) by mixing 100-200 µl of emulsion with 150 ml of dispersion water (Hydro S) and the result is shown in Figure 3B.

The order of drug entrapment efficiency values observed in the macroemulsions was found to be CUM processed by anti-solvent precipitation technique (80.03 ± 0.505) > CUM processed by modified vapor pressure diffusion method (75.63 ± 1.14) > unprocessed CUM (46.83 ± 0.24) (Table 6). Similarly, the amount of drug present at the oil-water interface of the macroemulsion also showed a higher percentage value for processed CUM-entrapped emulsions than the unprocessed drug-loaded emulsion. The observed higher drug entrapment and/or drug accumulation at the oil-water interface of the macroemulsions could be attributed/corroborated with the presence of an amorphous, pure crystalline or size-reduced form of the drug in the macroemulsion systems.

Macroemulsion stability

The appearance or disappearance of paired bean structures inside the macroemulsion is monitored by keeping the macroemulsion at 37º C (room temperature) and 25º C (refrigerator) over the period of 4 weeks. Figure 4 depicts the optical microscopic pictures taken at different weeks after the storage of macroemulsions at two different temperature conditions. The macroemulsions are stable until the third week of storage time at both temperature conditions, that too, by keeping the paired bean structure intact into them.

Therapeutic potential of macroemulsion

In vitro anti-inflammatory activity of macroemulsion containing the FEN or GIN

By following the method of Chandra et al. (2012) and adopted previously by Tamilvanan and Kaur (2016), the in vitro anti-inflammatory activity of unprocessed and processed FEN or GIN powder and 1ml of olive-and silicone-oils-based macroemulsions

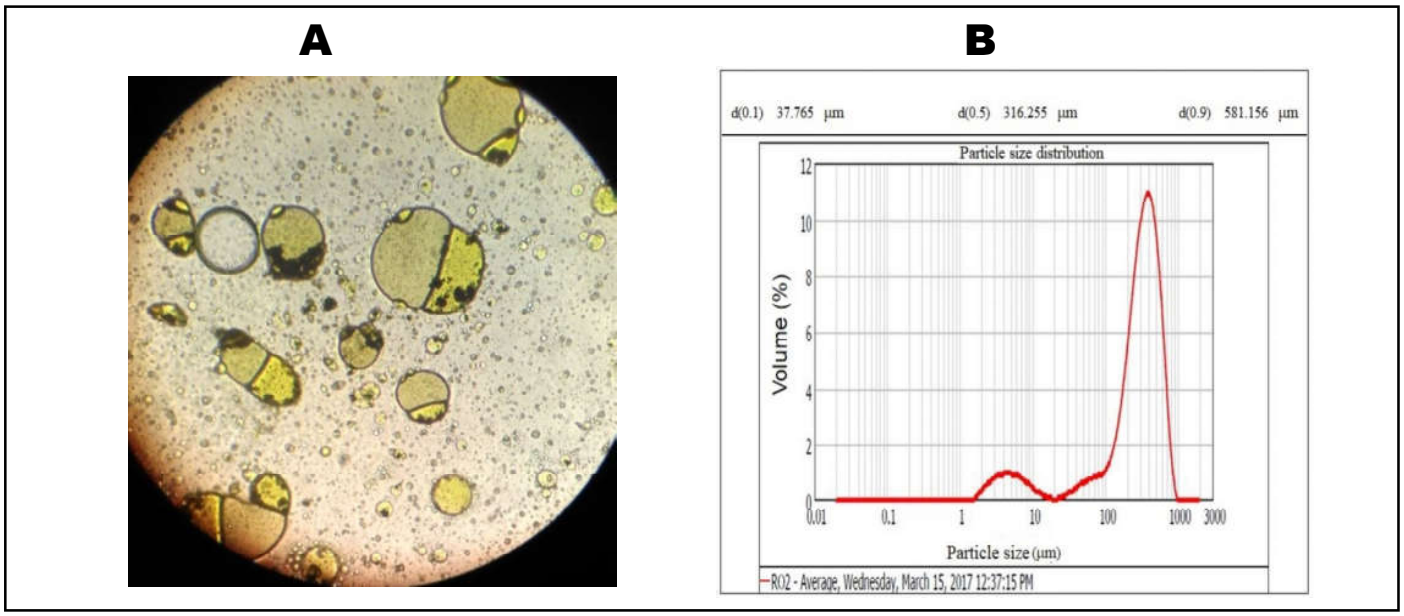

Figure 3. Optical microscopic pictures of curcumin-loaded macroemulsions containing the paired beans structure of dispersed oil droplets (A) and particle size analysis (d (0.5) = 316.255 µm) by Malvern Master-sizer (B).

Table 5. Typical formulas used to prepare macroemulsions containing unprocessed adapalene (ADP) and both processed and unprocessed curcumin (CUM), fenugreek (FEN) or ginger (GIN).

Drug name	Water phase		Oil phase	
	Water (g)	Tween 80 (g)	Olive oil (g)	Silicon oil (g)
Unprocessed ADP (40 mg)	9.782	0.408	1*	2
Processed and unprocessed CUM (40 mg)	19.56	0.40	1	4
Processed and unprocessed FEN (50-250 mg)	9.48	0.52	1	4
Processed and unprocessed GIN (50-250 mg)	9.48	0.52	1	4

* Castor oil (g), Processed means via modified vapor diffusion method or antisolvent precipitated method and unprocessed means natural structure of CUM, FEN or GIN

Table 6. Drug entrapment efficiency and drug amount at oil-in-water interface calculated for curcumin (CUM)-laden macroemulsions.

Compound CUM	Drug entrapment efficiency (%)	Drug amount at o/w interface (%)
Natural CUM	46.83	15.29
Modified vapour pressure diffusion method processed CUM	75.63	24.32
Anti-solvent precipitated method processed CUM	80.03	47.115

(equivalent to 9 mg of unprocessed or processed FEN or GIN powder) were determined.[28, 29] To avoid the use of laboratory animals for accessing the anti-inflammatory potential of drug-loaded macroemulsions, the protein denaturation assay can be used. Because the production of auto-antigens in certain inflammatory or arthritis diseases may be due to the denaturation of proteins in vivo.[30, 31] This test would be an indirect way of assessing the anti-inflammatory potentials of plant-derived active

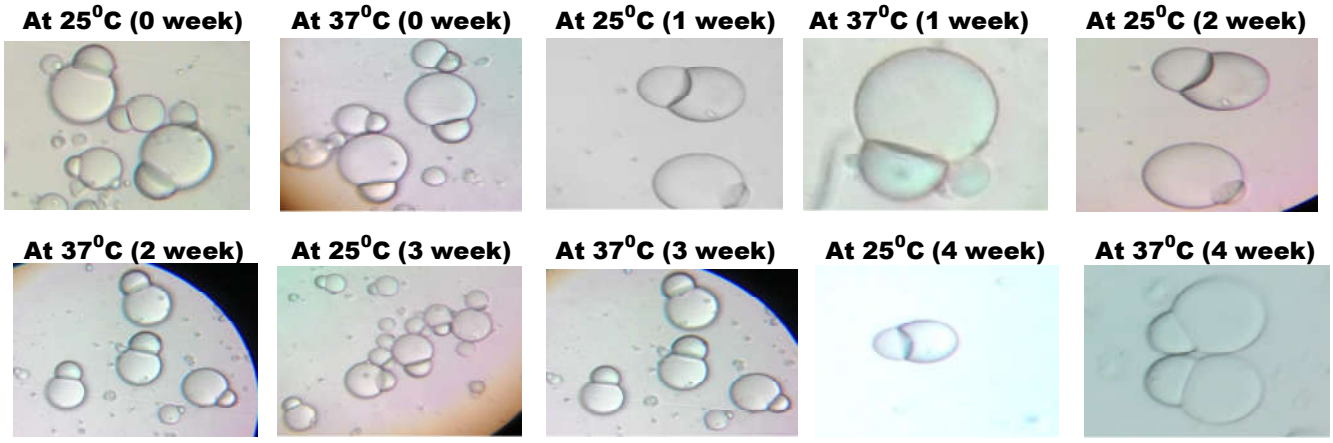

Figure 4 Optical microscopic pictures of macroemulsions stored at 25^0 and 37^0 C for 4 weeks.

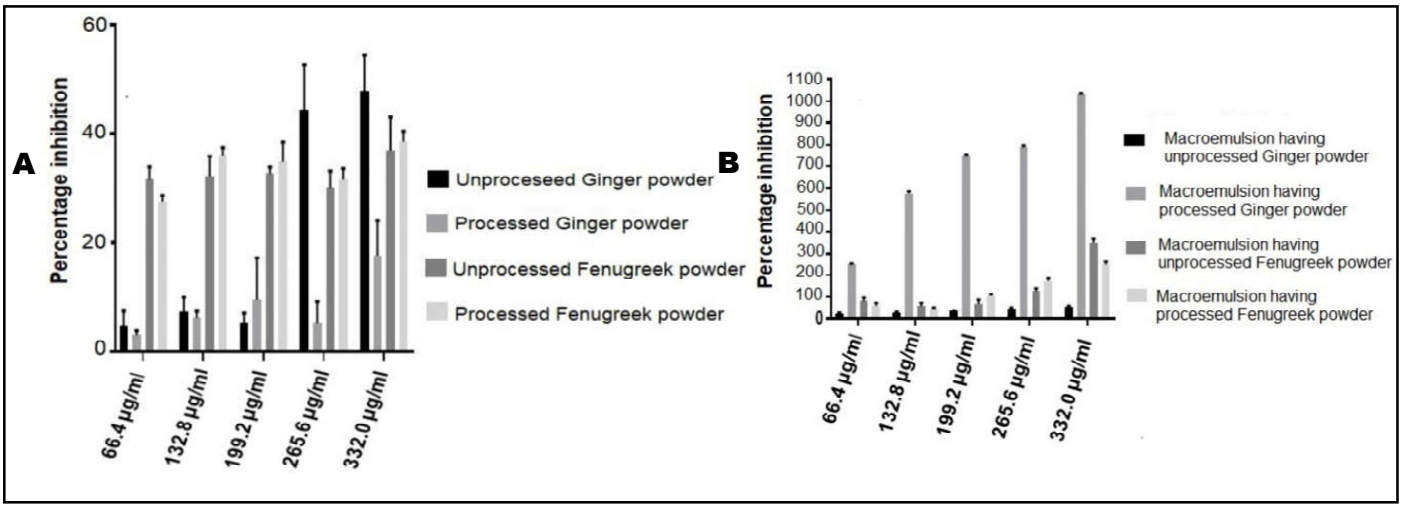

Figure 5. *In vitro* protein denaturation assay-based anti-inflammatory activities observed from (A) pH 7.4 phosphate buffer solutions containing unprocessed and processed fenugreek (FEN) or ginger (GIN) powder and (B) macroemulsions containing unprocessed and processed FEN or GIN powder at varying concentration levels (66.4, 132.8, 199.2, 265.6 and 332 µg/ml).

constituents, especially at their preliminary screening time.[28] The best formulation or good anti-inflammatory active constituents could be judged based on the higher percentage of protein inhibition at the lowest possible concentration level.

Figure 5 A&B depict the percentage inhibition values of protein denaturation reaction for Janus emulsions containing processed and unprocessed FEN or GIN (test formulations) and pH 7.4 phosphate buffer solutions containing processed and unprocessed FEN or GIN (control solutions). A concentration-dependent effect (from 66.4 to 332 µg/ml) on the percentage inhibition value of protein denaturation reactions at in vitro conditions was noticed. Furthermore, the percentage inhibition values for the protein denaturation reactions observed with test formulations were always higher than the values shown by controls. Therefore, macroemulsions containing the processed FEN or GIN could be of clinical interest for managing the inflammations produced at primary dysmenorrheal conditions.

Conclusion

The presence of API in two different physical forms/ states within the hydrophilic polymer-based microparticles influences the performances of API. The non-conventional application potential of hydrophilic polymer-based microparticles as an adjunct therapy is shown in this review. The compartmentalization made on the dispersed oil droplets of o/w macroemulsions put further be substantiated at the dispersed oil droplets in the nano-level category as well as incorporating the dual drug into the emulsion system to elicit a synergistic pharmacological activity for the management of other syndromes at reduced doses.

Future scope

The current review article explores the use and unique properties of hydrophobic eutectic liquid to

create microcapsule structures at in vitro conditions on contact with dissolution medium and thus the drug release retardation from the hydrophilic polymer matrix. Nevertheless, the microcapsule structure formation capability provided by the eutectic liquid needs to be tested at in vivo conditions after the insertion of hydrophilic polymer-based implants into the human body surfaces in the vicinity of body fluids. If it works, then, the in situ-forming microcapsules concept from hydrophilic polymers due to the presence of eutectic liquid needs further investigation. The current review also envisions the two-compartment structure in the dispersed oil droplets of macroemulsions. Such macroemulsions open the entrapment possibility of two different APIs possessing similar therapeutical activity but dissimilar physicochemical properties. Indeed, the exploration of two drugs-loaded macroemulsions for managing inflammatory bowel diseases, non-alcoholic fatty liver disease, etc., and inflammation-producing topical and ocular syndromes are currently undergoing in the research laboratories.

Acknowledgment

The funding provided by the Department of Biotechnology (DBT), Govt. of India under a Nanotechnology grant (Sanction No. 102/IFD/SAN/2383/2018-2019, Date: Sept 19, 2018) to the author Syed Nazrin Ruhina Rahman is greatly acknowledged.

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